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16. ABSTRACT

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KEY WORDS: Municipal wastewater sludge, mutagenicity, land application.

Introduction

Wastewater treatment plants in the United States process more than 8 billion gallons of municipal sewage each day (1). The treatment of municipal sewage results in the annual production of more than 6.8 million dry tons of sludge which must be disposed of in an economical and environmentally sound manner (2). Current practices employed for the disposal of sludge include landfilling, land application, ocean or fresh water dumping and incineration (3,4). It has been estimated that almost half of the municipal sludge in the United States is treated by some form of land disposal (2). Health hazards which may be associated with sludge disposal include pathogens (e.g., bacteria, viruses, protozoa, and helminths), trace elements (e.g., cadmium, lead, and zinc), nitrates and organics (5). While a great deal of information exists in the literature pertaining to the environmental fate of specific pathogens (6,7,8,9,10,11), heavy metals (12,13,14), and nitrate (15,16), much less is known about the fate and composition of organic chemicals in municipal sewage (5). A partial list of organic chemicals which have been identified in municipal sewage is provided in Table 1. Several of the chemicals listed in Table 1 have been found to induce increased frequencies of mutations in prokaryotic or eukaryotic systems (17,18). This paper presents the results of an on-going research project designed to measure the mobility and stability of organic mutagens in sludge amended soils. The results reported in this paper describe the mutagenic potential of twelve municipal sludges and one sludge amended soil. Additional information is currently being collected to better describe the stability and mobility of sludge-borne mutagens as well as the

potential for plant uptake of mutagens from soil.

Procedures

Sludge Samples-Twelve municipal wastewater sludge samples were initially collected from seven cities with populations ranging from 45,000 to 2,500,000. The gross characteristics of the sludges are provided in Table 2. In order to preserve the confidentiality of the sludge sources, each sludge was given a two letter identification code; multiple samples collected from a single source are followed by a number. Sludges collected in the southern United States include SMO, SM1, SM2, SM3, JT1, JT2, VG, BN; sludges collected from the northwest United States include ST, TA, SK, and RD. The approximate industrial contribution to each sludge was SMO and SM1-19%, JT1 and JT2-6%, VG-20%, BN-50%, SM2 and SM3-30%; the amount of industrial contribution for ST, TA, SK, and RD were not provided.

Soil-For the initial studies to investigate the effect of land application on sludge-bound mutagens, barrel-sized lysimeters of a Padina loamy sand were collected. The procedures for the collection of undisturbed soil monolith lysimeters are described in Brown et al. (18). The characteristics of the Padina soil are as follows: cation exchange capacity - 4.3 meg/100g; total organic carbon - 0.4%; bulk density - 1.38 g/cm^3; available water - 7.8% (calculated as the difference between moisture content at -1/3 bar and -15 bars). The SB3 sludge was applied to the surface to 15 cm soil at a rate of 100 mT/ha (11 kg per lysimeter). This high loading rate was selected to

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*Mention of brand names do not constitute an endorsement.

optimize the potential for detecting mutagens in the sludge-amended soil.

Extraction-The sludge and sludge-amended soil samples were sequentially extracted with methylene chloride and methanol (Reagent grade, Fisher Chemicals, Fairlawn, NJ) using a Soxhlet apparatus or a Tecator* Soxtec Apparatus (Tecator, Hoganas, Sweden). To eliminate any interferences during sample processing due to the varying moisture contents of samples, sludge and soil samples were dried at 60°C for 24 hr prior to extraction. This should not have significantly altered the final composition of the sludge extracts, as the Tecator extractions were conducted at 130°C or above. For the initial eleven sludge samples, fifteen to twenty grams of dried sludge was combined with an equal weight of anhydrous sodium sulfate (anhydrous granular, J. T. Baker Chemical Co., Phillipsburg, NJ), weighed into a cellulose extraction thimble and mixed with a glass rod. The thimble and glass rod were placed in a 20 ml capacity Soxhlet extractor. Condensers and boiling flasks containing 200 ml of solvent were assembled with the extractorand placed on the heater apparatus. Refrigerated coolant was circulated through the condensers. A 30 ml volume of solvent was introduced into the extractor from the top to facilitate flushing of the extractor chamber. Heat was applied to the boiling flask to initiate a 6 hour solvent extraction of the organic components; the temperature was adjusted to give six flushings per hour.

The SB3 and sludge-amended soils were extracted using the Tecator Soxtec apparatus. Samples were prepared and added to cellulose extraction thimbles following the same procedures as for the Soxhlet apparatus. The thimbles were then placed in the thimble support and

attached to the thimble holder. The glass cups were filled with 25 ml of methylene chloride or methanol and clamped into the condensors. Each sample was extracted for 5 min in the boiling position and 55 min in the rinsing position. After the rinsing time was completed, the condenser valves were closed and the residual solvent evaporated. Each dried sample was then separated into two fractions, methylene chloride and methanol; the residue was then weighed, redissolved in dimethyl sulfoxide, and an aliquot of each fraction combined in equal proportions to represent a third fraction. All extractions were conducted in triplicate.

Bioassay-The Salmonella/microsome assay of Ames (20) was used to evaluate the mutagenicity of the solvent extractable organic compounds in sludge and sludge-amended soil. The Salmonella strains were kindly supplied by Dr. Bruce N. Ames (University of California, Berkeley, CA). The procedural methods were the same as Ames (20) with the modifications suggested by Maron and Ames (21). The Salmonella tester strains TA98 and TA100 were utilized with and without metabolic activation. The S9 mixture (S9 = 9,000 x g supernatent from homogenized rat liver) contained 0.3 ml rat-liver S9 and 0.7 ml cofactor supplement (11.4 mM $MgCl_2$, 47 mM KCl, 7.1 nM glucose-6-phosphate, 5.7 mM NADP and 140 mM potassium phosphate buffer (at pH 7.4). Aroclor-induced Sprague-Dawley rat liver was obtained from Litton Bionetics (Charleston, SC). Samples were tested with an unusually high level of metabolic activation because previous research with hazardous waste (22) and synthetic fuel (23) has indicated that for complex mixtures, optimum results are obtained at high levels of activation. Each of three fractions obtained from the sludge or

sludge-amended soil was tested in the standard plate incorporation assay on duplicate plates in two independent experiments (N=4) at a minimum of 5 dose levels (mg/plate): 0.5, 1.0, 2.5, 5, and 10. The data were analyzed using the modified 2-fold rule (24). Using this procedure, a response is considered positive if the average response for at least two consecutive dose levels was greater than twice the average response for the concurrent negative/solvent control (24).

Both strains were checked monthly for nutritional markers (histidine and biotin) and other genetic characteristics, i.e., sensitivity to crystal violet, ampicillin, and UV light. On each test date, all tester strains were calibrated with positive and negative controls. Positive controls included 1 µg/plate sodium azide (Sigma, St. Louis, MO) for TA100 and 25 µg/plate 2-nitrofluorene (Aldrich, Milwaukee, WI) for TA98. 2-Aminoanthracene (Sigma) at 5 µg/plate was used with both strains to verify the functioning of the metabolic activation system. All reagents and extracts were tested for sterility; dimethyl sulfoxide (DMSO, Grade I Sigma) was used as a solvent control as suggested by Maron and Ames (21).

Results and Discussion

A total of 36 fractions from 12 municipal wastewater sludges were extracted for testing in the bioassay. All of the sludge fractions were tested in strain TA98 and six fractions were tested in both strain TA98 and TA100. None of the fractions tested induced a positive response in strain TA100 with or without metabolic activation (data not shown). The data presented in Table 3 are the dose-response and concurrent solvent control values for each of the sludge fractions

tested in strain TA98. These data provide a description of the mutagenic potential of the residual organics in each sludge fraction. The sludge fractions were tested at relatively high dose levels (up to 10 mg per plate) because much of the solvent extractable organic material was believed to be inert or non-reactive in the microbial bioassay. The sludge fractions displayed a tremendous amount of variability in their ability to induce TA98 mutations. While only indirect-acting mutagens were detected in the majority of sludge fractions, direct acting mutagens were detected in one sample; and, none of the three fractions from sludge samples JT1, BN, RD, and ST induced a positive response (Table 3).

Chemicals contained in the methylene chloride extractable organics should represent the more non-polar sludge components. Sludge samples for which the methylene chloride fraction failed to induce a doubling of revertants at two consecutive dose levels include the SM2, JT1, BN, RD, ST, and JT2 sludges. At a dose level of 5 mg per plate with activation, the methylene chloride fraction of SMO and SM1 induced 74 and 92 total revertant colonies or slightly greater than two times background (Table 3). The fourth sample from the SM plant, SM3, induced 151 total revertant colonies (4.7 times background) at a dose level of 10 mg per plate without activation. In the presence of metabolic activation, the mutagenic response induced by the methylene chloride fraction of the SM3 sludge was reduced or completely eliminated. The maximum mutagenic response induced by a methylene chloride fraction with metabolic activation was 155 total revertants (4.4 times background) induced by the VG sludge at a dose of 10 mg per plate. Thus, the mutagenic potential of the methylene

chloride fractions extracted from twelve different sewage sludges ranged from a low of 3 net revertants to a high of 119 net revertants.

The solvent extractable organics contained in the methanol fractions should include the majority of the polar or water soluble chemicals. The methanol fraction from seven of the twelve sludges, including SMO, SM1, JT1, BN, ST, and TA failed to induce a doubling of revertant colonies at two consecutive dose levels (Table 3). At a dose level of 10 mg per plate with metabolic activation, the methanol fraction of the SK sludge induced 65 total revertants (2.1 times background) while the JT2 sludge induced 77 total revertants (2.5 times background). The maximum mutagenic response observed in a methanol fraction was 119 total revertants (3.4 times background) induced by the VG sludge at a dose of 5 mg per plate with activation (Table 3). For the methanol fractions, the response induced in the bioassays ranged from 2 net revertants to 87 net revertants. In addition, for all twelve sludges tested, a mutagenic response was observed with the methanol fraction only in the presence of metabolic activation.

The response induced by the combined fraction provides an estimation of the mutagenic potential of the total organic carbon of sewage sludge. The results are only an estimate because the two fractions do not represent the entire organic fraction of the sludge. However, the results obtained from the combined do proivde an indication of the potential interactions of the components of the methylene chloride and methanol fractions. Eleven of the twelve combined fractions tested failed to induce a doubling of revertant colonies at two consecutive dose lvels. At a dose level of 10 mg per

plate, the combined fraction of the SM3 sludge induced 80 net revertants (4.0 times background) without activation and 71 net revertants (2.7 times background with activation (Table 3). These results indicate that for the majority of sludge samples tested, the combined methylene chloride and methanol fractions did not induce a mutagenic response in the bioassay.

The weighted activity of the fractions from ten of the sludges is presented in Figure 1. This value was obtained by multiplying the specific activity (net revertants mg^{-1}) from Table 3 times the residue weight (mg g^{-1}) for each fraction and allows for a comparison of sludge samples based on equivalent weights. The results indicate that four of the ten sludges (SM0, VG, BN, and TA) contained relatively low quantities of organic mutagens. Although the specific activity of the fractions of the GC sludge were high, the weighted activity is relatively low due to the low quantity of solvent extractable organics. Moderate levels of organic mutagens were detected in the sludges from JT1, RD, ST, and SK. The combined fraction of the SM1 sludge contained more than 800 net revertants per gram sludge extracted (Figure 1); and the methanol fraction of the SM2 sludge appears to contain the greatest quantity of organic mutagens inducing and estimated 1012 net revertants per gram of sludge. Thus, the quantitative comparison of sludge mutagens indicates that the SM1 and SM2 sludges contained the greatest quantities of organic mutagens.

A third dry sludge sample was collected from the SM wastewater treatment plant for use in a land application experiment. The results from the analysis of SM3 sludge amended soils for the first 28 days after sludge application are presented in Figures 2 to 5. For the

methylene chloride fraction of the SM3 sludge or sludge-amended soil, an increased mutation frequency was observed without metabolic activation, but not with activation. There was no appreciable difference in the mutagenic potential of the methylene chloride fraction from sludge or sludge-amended soil collected immediately after and 28 days following sludge application (Figure 2). For the methanol fraction, mutagenic activity was detected only in the presence of metabolic activation. At a dose of 10 mg per plate with activation, the methanol fraction from the sample collected immediately after sludge application induced less than 60 net revertants, while the sample collected 28 days later induced almost 169 net revertants (Figure 3), and the sample collected 56 days after sludge application induced 169 net revertants (data not shown).

The combined fraction represents the total methylene chloride and methanol extractable organics and should most accurately resemble the residual mutagenicity of the sludge amended soil. In the Padina sand, low levels of mutagenic activity were observed in the samples collected immediately following and 28 days after waste application without metabolic activation (Figure 4). In the presence of metabolic activation, the response increased from less than 40 net revertants immediately after application to almost 150 and 130 net revertants 14 and 28 days after application, respectively at a dose level of 10 mg per plate (Figure 4). Thus, the response observed in the combined fraction from the Padina sand with metabolic activation very closely mimicked that observed in the methanol fraction with activation.

The results presented in Figure 5 depict the interactions which may occur between the fractions of the sludge amended soil. Without metabolic activation, the methanol fraction did not induce a significant increase in revertant colonies, while the response induced by the combined fraction was consistently less than that induced by the methylene chloride fraction and followed the same general trend over time (Figure 5). These results indicate that the methanol fraction slightly inhibited the mutagenic components of the methylene chloride fraction when tested as a combined fraction without metabolic activation. In the presence of metabolic activation, there was no appreciable difference between the response induced by the methanol and the combined fraction; and, the highest level of mutagenic activity was observed in the methanol fraction collected 28 days after sludge application (Figure 5). Thus, the methylene chloride fraction had little or no effect on the mutagenic potential of the methanol fraction when tested as a combined fraction with metabolic activation.

The results of the present study indicate that municipal sewage sludges are likely to contain at least low levels of mutagenic chemicals. While several of the sludges evaluated had no detectable mutagenic constituents, others had a relatively high mutagenic potential. Previous research by Tabor *et al.* (25) and Hopke *et al.* (26) also reported large variations in the mutagenic potential of different samples of municipal waste and isolated specific samples containing high levels of mutagenic activity. The maximum specific activity of a sludge fraction observed in the present study was 120 net revertants at a dose of 10 mg per plate with activation, while the maximum weighted activity was 1012 revertants per gram. Four of five

sludge samples tested by Hopke *et al*. (27) failed to induce a positive response, while the specific activity of the fifth sample was 3300 net revertants at a dose of 4.17 mg per plate with metabolic activation. In addition, Angle and Baudler (28) suggest that degradation reduced the mutagenic potential of sludge amended soil within 7 days after application. The analysis of the sludge amended soil indicates that the mutagenic potential of the residual organics was increased with time. A study by Johnson and Baker (29) also observed an increase in the mutagenic potential of sludge amended soil. This increased activity may be a result of biodegradation of non-mutagens into mutagenic compounds, biodegradation of inert organics resulting in a greater expression of the residual mutagens, or some type of synergistic interactions between the residual mutagens in the soil. The results of the present study indicate that some sludges may persist in the soil for extended periods of time and that further research is needed to evaluate the potential for leaching or plant uptake of sludge-borne mutagens.

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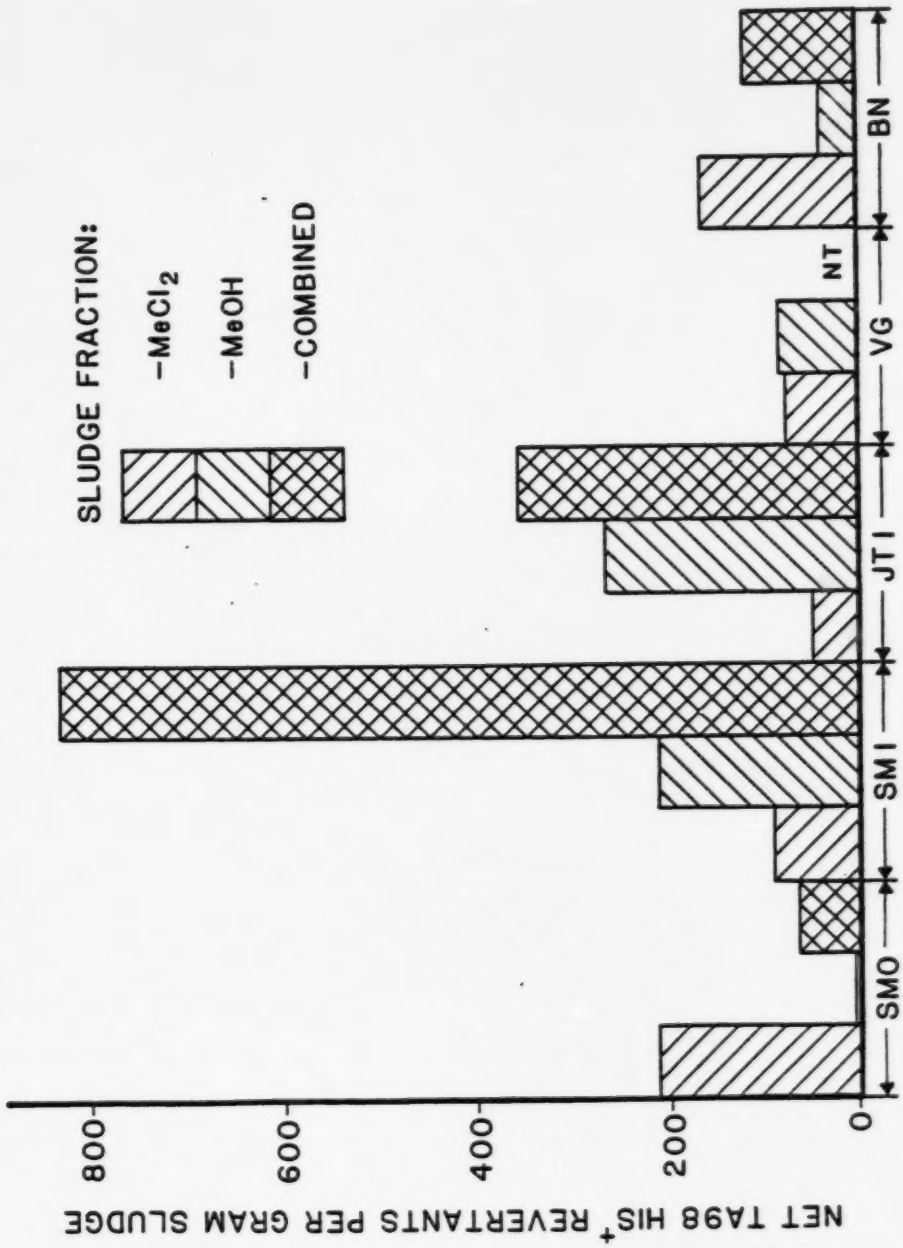
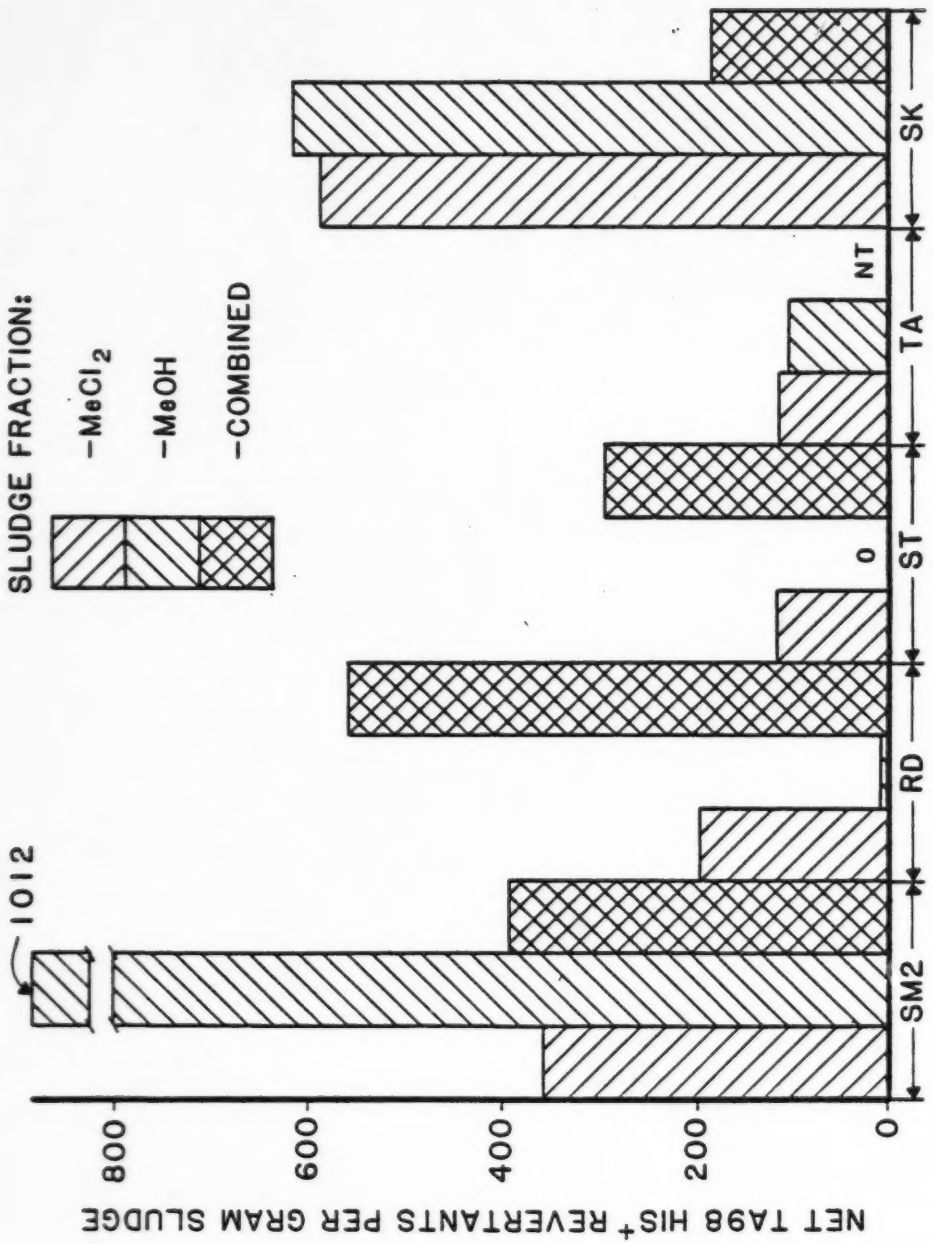


Figure 1. Weighted activity (net revertants.mg^{-1} x mg.g^{-1}) of solvent extractable organic compounds in samples of sewage sludge.



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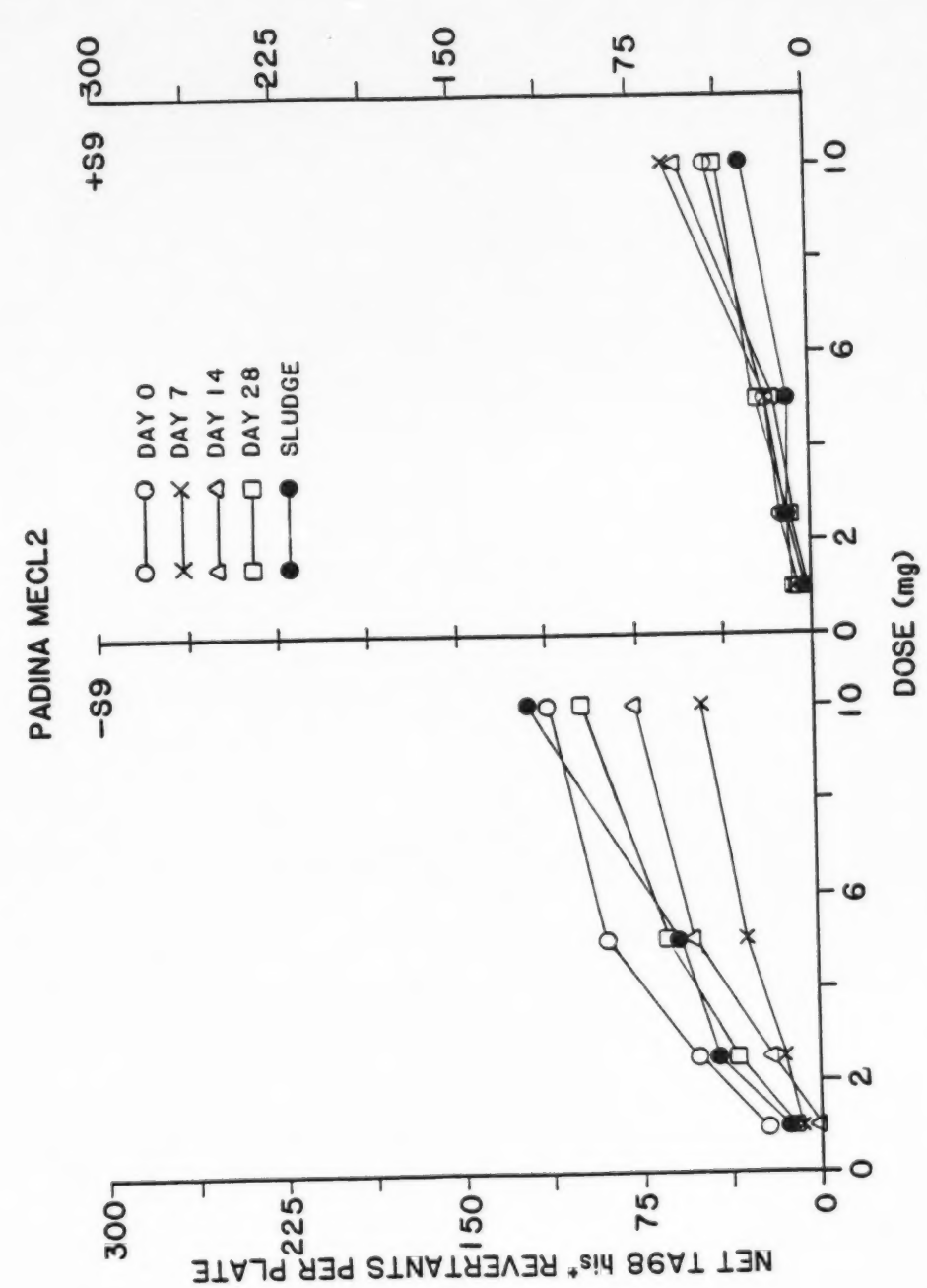

Figure 2. Mutagenic potential of methylene chloride extractable organics from SM3 sludge amended Padina sand.

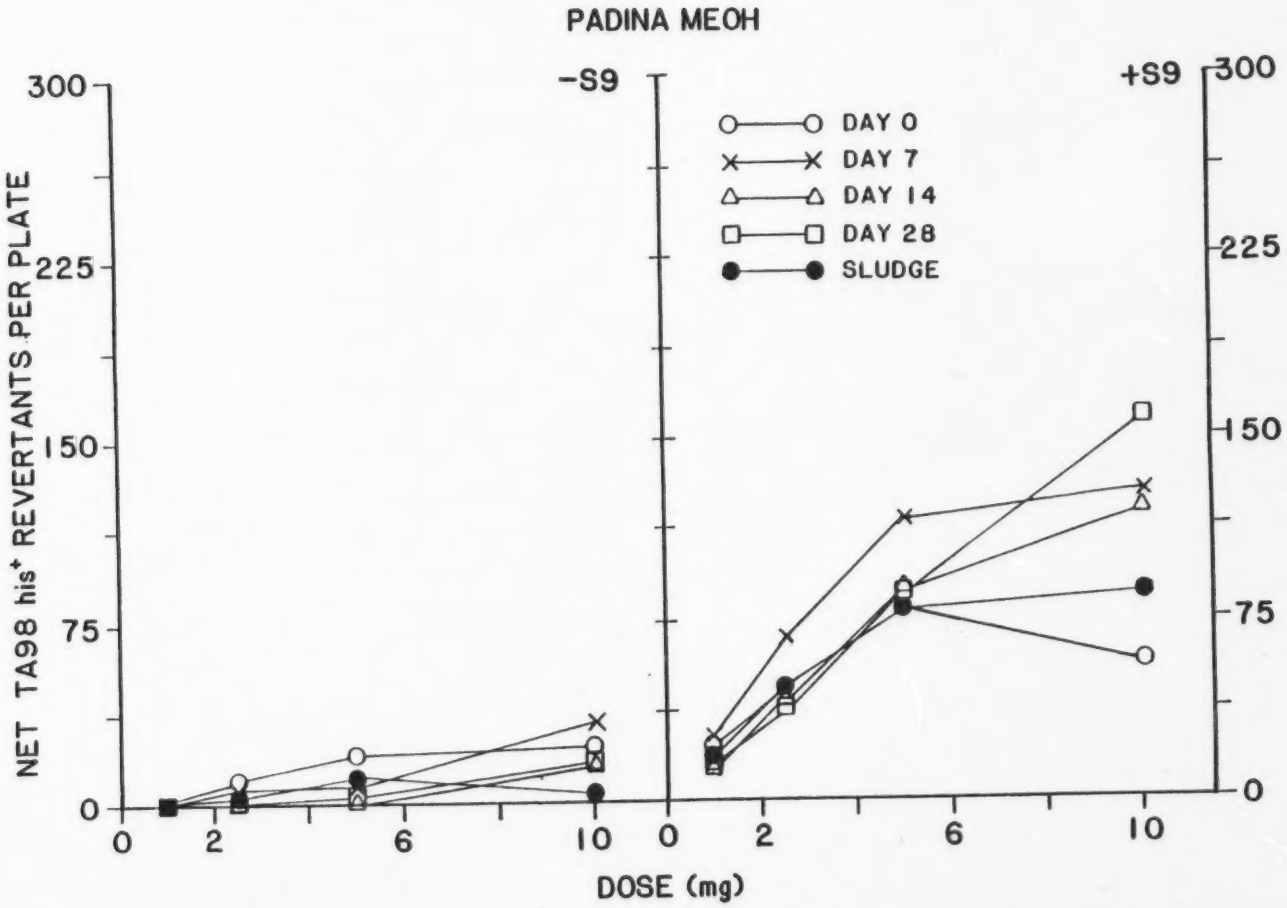

Figure 3. Mutagenic potential of methanol extractable organics from SM3 sludge amended Padina sand.

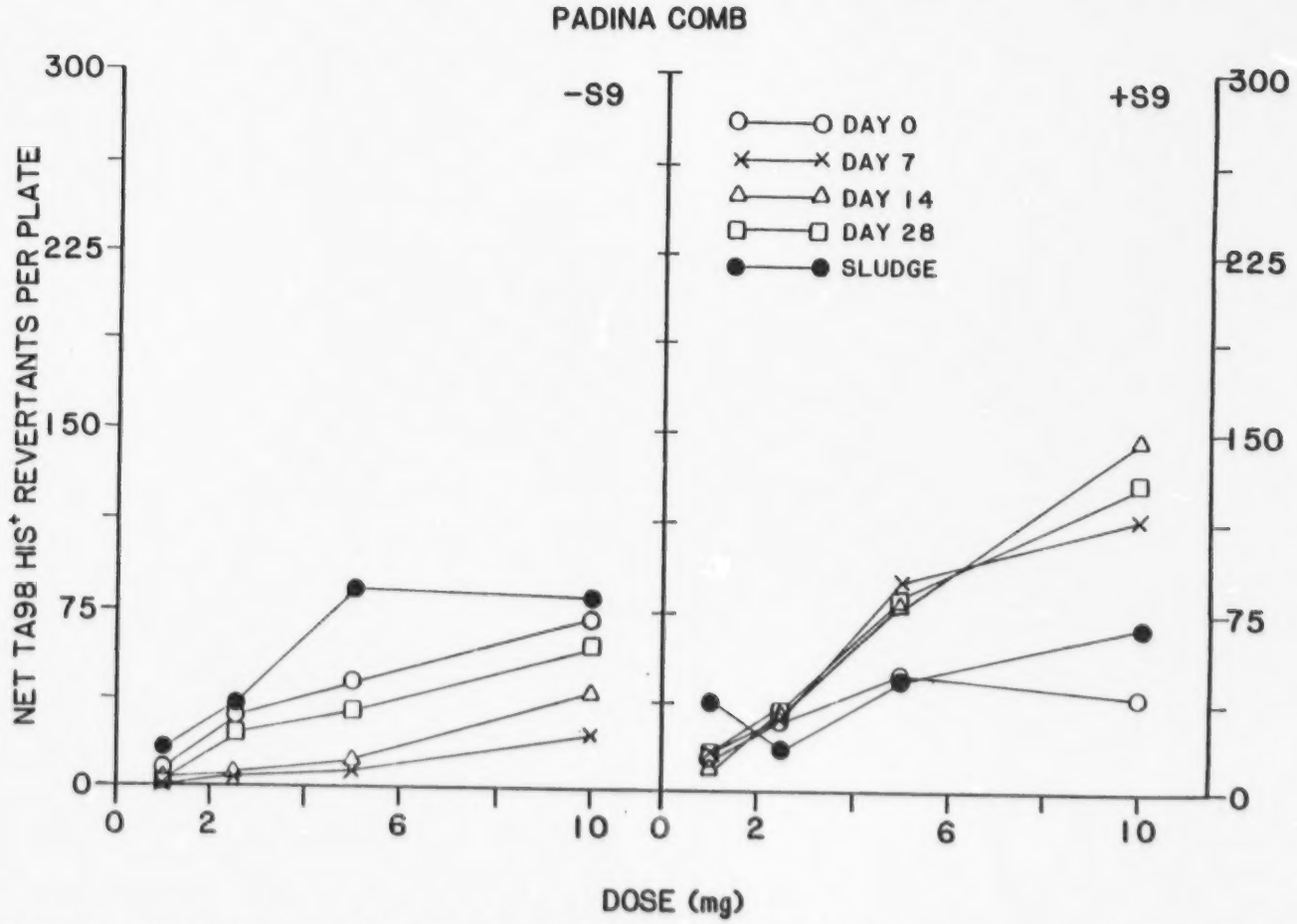

Figure 4. Mutagenic potential of combined fraction from SM3 sludge amended Padina sand.

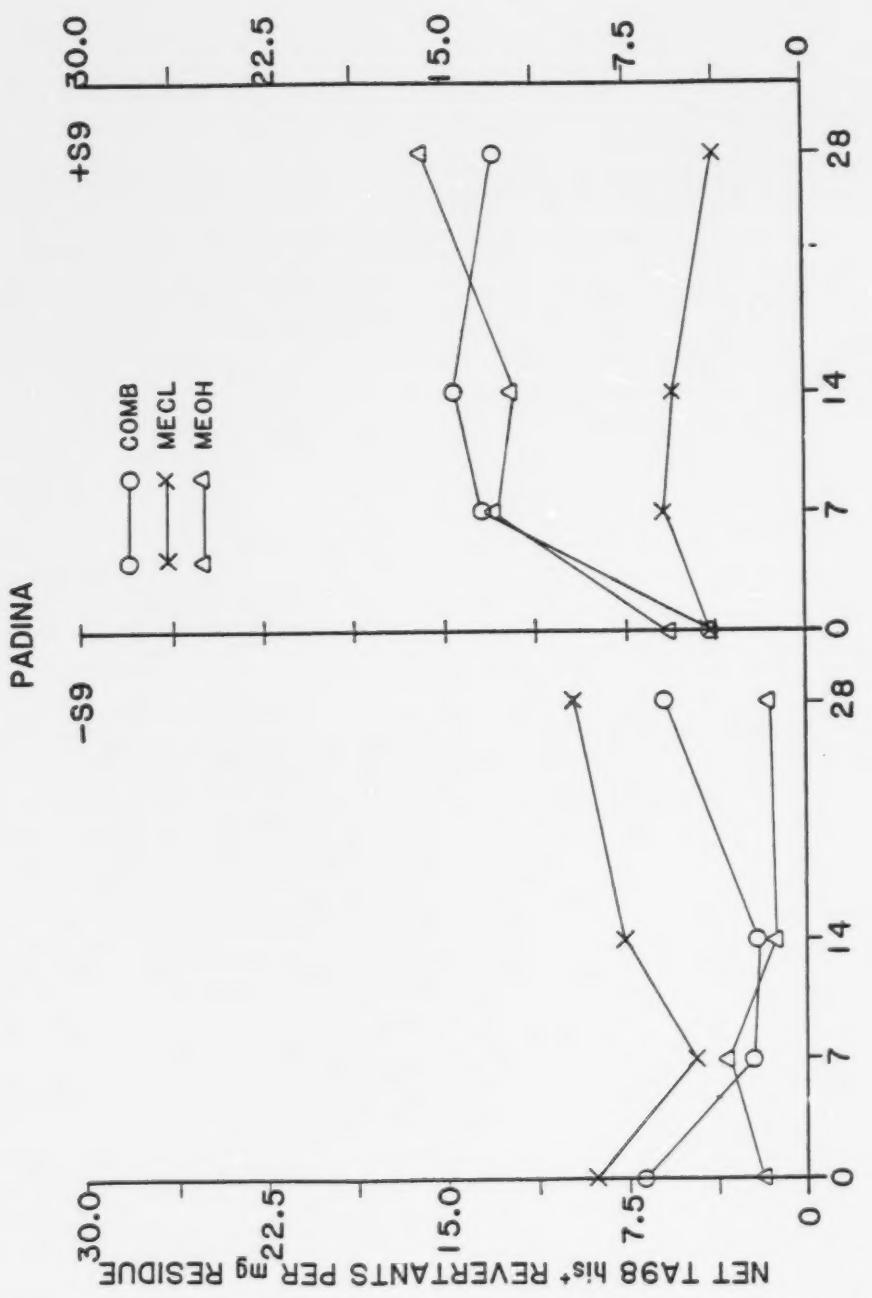

Figure 5. Mutagenic potential of three fractions extracted from SM3 sludge amended Padina sand.

TABLE 1. Partial List of Organic Compounds Identified in Samples of Sewage Sludge.

Compound	Concentration Range (mg/kg)	Carcino-genicity[1]	Muta-genicity[2]	Muta-genicity[3]	Reference
Acrylonitrile	.04-82	+	+	+	Jacobs et al. (30)
Chlorobenzene	2.1-846	0	0	+	"
p-chlorotoluene	1.1-324	0	0	0	"
1,2-dichlorobenzene	.03-809	0	0	+	"
1,3-dichlorobenzene	.02-1650	0	0	+	"
Ethylbenzene	1.2-66	0	0	0	"
Hexachloroethane	.0004-17	+	0	0	"
Styrene	.12-5850	0	-	0	"
Tetrachloroethane	.00001-0.12	+	-	-	"
1,2,3-trichlorobenzene	.006-51.2	0	0	+	"
1,3,5,trichlorobenzene	.005-39.7	0	0	+	"
Aldrin	.013-0.1	0	-	0	Saleh et al. (31)
Dieldrin	.003-.032	+	-	0	"
DDT	.006-.011	+	-	0	"
DDE	.011	+	-	0	"
DDD	.006-.048	+	-	0	"
2,4-D	7.6-41	0	0	+	"
Dieldrin	<03-0.44	+	-	0	Furr et al.. (32)
PCB's	<.01-23	+	-	+	"
Fluoranthene	1-334	-	-	-	Bridle and Webber (33)
Fluorene	0.8-115	0	0	0	"
Benzo(a)pyrene	34	+	+	+	"
Anthracene	8-311	0	0	0	"
Chrysene	<600	+	+	+	Webber et al. (34)
Pyrene	<600	-	-	-	"
Benzo(a)pyrene	<600	+	+	+	"
Dibutylphthalate	60-500	+	+	+	Strachan et al. (35)

TABLE 1 continued.

Compound	Concentration Range (mg kg)	Carcinogenicity[1]	Mutagenicity[2]	Mutagenicity[3]	Reference
Di(2-ethylhexyl)-phthalate	30-130	+	-	0	Strachan et al. (35)
Lindane	.006-.003	+	-	0	Majeti and Clark (36)
Heptachlor	.001-.021	+	-	0	"
Chlordane	.008-.030	0	0	0	"
PCB	.07-1.1	+	-	+	"

1 - carcinogencity as determined from limited review of literature. + = positive; - = negative; 0 = unknown.
2 - mutagenicity as measured in standard plate incorporation with S. typhimurium. + = positive; - = negative; 0 = unknown.
3 - foreward mutations as measured in methionine assay with A. nidulans. + = positive; - = negative; 0 = unknown.

TABLE 2. Gross Characteristics of Sewage Sludge Samples

Sample	% H_2O	% Solids	% Ash	Organic Matter Composition[1]		
				COM	SOM	BOM
SMO	87	13	5.7	7.3	2.1	5.1
SM1	10	90	36.0	54.0	7.6	46.0
JT1	87	13	5.5	7.4	4.5	3.0
VG	30	70	64.0	6.0	0.6	5.4
BN	68	32	24.0	7.9	3.8	4.1
SM2	15	85	31.0	54.0	12.0	32.0
JT2	16	84	37.0	47.0	9.0	38.0
ST	78	32	15.0	17.0	10.4	6.6
TA	85	15	10.4	4.6	2.4	2.2
SK	82	18	10.2	7.8	8.6	-
RD	72	28	14.0	14.0	3.3	10.7

1. Organic matter composition (%)
   COM = Combustible organic matter
   SOM = Total solvent extractable organic matter (methanol and methylene chloride)
   BOM = Bound organic matter (COM-SOM).

TABLE 3. Mutagenicity of Solvent Extractable Organic Compounds from Various Samples of Sewage Sludge.

Sample	Dose mg/plate	Total TA98 his^+ Revertants (Mean ± SD)					
		$MeCl_2$		MeOH		Combined	
		-S9	+S9	-S9	+S9	-S9	+S9
SMO	0.0	32 ± 4.8	32 ± 6.2	34 ± 3.7	40 ± 11.0	34 ± 3.7	40 ± 12.0
	0.1	16 ± 18.0	49 ± 16.0	14 ± 7.2	33 ± 2.4	25 ± 2.9	NT*
	0.5	29 ± 6.4	44 ± 12.0	30 ± 8.8	36 ± 7.4	25 ± 3.9	34 ± 3.4
	1.0	33 ± 3.2	60 ± 11.0	30 ± 2.1	41 ± 15.0	30 ± 9.1	30 ± 8.1
	2.5	35 ± 8.2	55 ± 16.0	35 ± 5.1	36 ± 3.9	31 ± 6.7	33 ± 6.2
	5.0	38 ± 7.2	74 ± 5.1	36 ± 5.1	42 ± 3.9	51 ± 7.8	37 ± 11.0
	10.0	47 ± 6.6	70 ± 13.0	35 ± 8.0	22 ± 8.3	21 ± 5.5	32 ± 9.4
SM1	0.0	32 ± 5.7	37 ± 5.8	32 ± 3.3	37 ± 4.2	32 ± 3.3	37 ± 4.2
	0.1	35 ± 7.8	41 ± 5.3	38 ± 7.1	43 ± 9.5	32 ± 5.8	40 ± 7.8
	0.5	41 ± 13.0	43 ± 5.1	32 ± 6.8	44 ± 8.9	37 ± 7.9	41 ± 4.8
	1.0	46 ± 21.0	44 ± 6.1	39 ± 5.6	36 ± 1.5	39 ± 8.2	52 ± 8.0
	2.5	44 ± 6.0	43 ± 5.5	33 ± 6.3	50 ± 10.0	38 ± 7.3	62 ± 16.0
	5.0	44 ± 2.6	92 ± 6.7	41 ± 7.9	73 ± 14.0	45 ± 11.0	98 ± 9.4
	10.0	52 ± 12.0	80 ± 22.0	23 ± 11.0	94 ± 10.0	20 ± 4.3	49 ± 2.0
SM2	0.0	32 ± 4.5	29 ± 9.4	32 ± 5.3	37 ± 3.7	32 ± 3.8	31 ± 12.0
	0.1	23 ± 3.6	31 ± 4.5	NT	NT	21 ± 8.8	24 ± 8.2
	0.5	24 ± 5.7	31 ± 8.1	30 ± 4.1	54 ± 20.0	20 ± 4.6	32 ± 5.7
	1.0	26 ± 11.0	38 ± 10.0	30 ± 12.0	58 ± 26.0	20 ± 6.8	37 ± 3.2
	2.5	NT	NT	31 ± 5.7	89 ± 54.0	NT	NT
	5.0	42 ± 24.0	47 ± 21.0	36 ± 6.0	115 ± 32.0	29 ± 10.0	33 ± 13.0
	10.0	43 ± 49.0	119 ± 11.0	49 ± 12.0	71 ± 45.0	41 ± 5.0	36 ± 10.0
SM3	0.0	32 ± 1.9	41 ± 2.7	33 ± 4.8	48 ± 7.0	27 ± 5.0	43 ± 12.0
	0.5	34 ± 12.0	39 ± 7.0	33 ± 3.0	52 ± 8.0	35 ± 13.0	57 ± 7.0
	1.0	44 ± 8.5	40 ± 11.0	34 ± 3.0	63 ± 9.0	45 ± 20.0	81 ± 17.0
	2.5	75 ± 5.7	52 ± 8.2	36 ± 5.0	97 ± 29.0	60 ± 16.0	78 ± 27.0
	5.0	84 ± 17.0	49 ± 8.4	43 ± 11.0	126 ± 48.0	110 ± 21.0	86 ± 16.0
	10.0	151 ± 14.0	66 ± 13.0	35 ± 3.0	135 ± 15.0	107 ± 13.0	114 ± 26.0

TABLE 3 continued.

Sample	Dose mg/plate	Total TA98 his+ Revertants (Mean ± SD)					
		$MeCl_2$		MeOH		Combined	
		-S9	+S9	-S9	+S9	-S9	+S9
JT1	0.0	32 ± 3.3	37 ± 4.2	32 ± 3.3	37 ± 4.2	32 ± 3.2	37 ± 4.2
	0.1	34 ± 8.0	35 ± 9.7	14 ± 16.0	38 ± 7.6	31 ± 4.7	32 ± 3.8
	0.5	32 ± 8.1	43 ± 16.0	36 ± 8.4	30 ± 4.8	30 ± 3.8	36 ± 5.6
	1.0	25 ± 16.0	47 ± 2.4	37 ± 8.2	28 ± 4.9	33 ± 6.1	45 ± 5.1
	2.5	33 ± 8.1	53 ± 8.9	36 ± 7.8	36 ± 12.0	30 ± 7.8	44 ± 5.1
	5.0	47 ± 10.0	53 ± 35.0	30 ± 4.8	41 ± 8.5	48 ± 11.0	26 ± 18.0
	10.0	15 ± 2.5	32 ± 4.6	20 ± 4.0	29 ± 7.0	NT	56 ± 10.0
VG1	0.0	29 ± 3.1	35 ± 7.1	29 ± 3.1	35 ± 7.1	NT	NT
	0.1	28 ± 7.5	24 ± 7.0	25 ± 9.6	33 ± 11.0	NT	NT
	0.5	29 ± 7.8	38 ± 19.0	30 ± 6.2	42 ± 12.0	NT	NT
	1.0	30 ± 17.0	44 ± 14.0	36 ± 14.0	63 ± 14.0	NT	NT
	2.5	25 ± 3.9	50 ± 14.0	35 ± 22.0	82 ± 9.5	NT	NT
	5.0	35 ± 10.0	93 ± 14.0	52 ± 15.0	119 ± 17.0	NT	NT
	10.0	NT	155 ± 11.0	NT	NT	NT	NT
BN	0.0	29 ± 3.1	35 ± 7.1	29 ± 3.1	35 ± 7.1	29 ± 3.1	35 ± 7.1
	0.5	30 ± 8.5	45 ± 10.0	34 ± 1.7	43 ± 13.0	33 ± 6.2	45 ± 11.0
	1.0	40 ± 3.3	51 ± 15.0	30 ± 9.1	42 ± 10.0	33 ± 14.0	51 ± 13.0
	2.5	38 ± 6.1	61 ± 13.0	34 ± 9.9	41 ± 8.6	30 ± 15.0	47 ± 12.0
	5.0	35 ± 6.3	52 ± 15.0	38 ± 14.0	56 ± 21.0	36 ± 12.0	59 ± 19.0
	10.0	45 ± 7.9	83 ± 12.0	41 ± 7.4	58 ± 16.0	37 ± 8.5	74 ± 7.2
RD	0.0	31 ± 4.3	31 ± 4.6	31 ± 4.3	31 ± 4.6	32 ± 5.3	37 ± 3.7
	0.5	23 ± 8.8	41 ± 18.0	23 ± 6.6	25 ± 6.9	32 ± 5.9	39 ± 17.0
	1.0	26 ± 2.5	42 ± 4.3	25 ± 5.2	32 ± 7.3	27 ± 7.7	57 ± 14.0
	2.5	28 ± 8.2	39 ± 26.0	25 ± 9.2	29 ± 8.1	23 ± 5.3	71 ± 5.9
	5.0	NT	51 ± 13.0	21 ± 5.3	38 ± 13.0	NT	47 ± 36.0
	10.0	NT	NT	27 ± 8.8	50 ± 19.0	NT	Tox**

TABLE 3 continued.

Sample	Dose mg/plate	Total TA98 his+ Revertants (Mean ± SD)					
		$MeCl_2$		MeOH		Combined	
		-S9	+S9	-S9	+S9	-S9	+S9
ST	0.0	36 ± 1.6	32 ± 7.7	36 ± 1.6	32 ± 4.7	25 ± 5.7	29 ± 3.0
	0.5	31 ± 4.3	35 ± 12.0	39 ± 18.0	39 ± 6.7	24 ± 4.5	32 ± 10.0
	1.0	35 ± 5.0	35 ± 9.0	32 ± 14.0	42 ± 12.0	23 ± 7.4	29 ± 5.2
	2.5	34 ± 5.4	33 ± 6.0	27 ± 13.0	28 ± 2.2	29 ± 14.0	40 ± 11.0
	5.0	34 ± 12.0	35 ± 14.0	32 ± 13.0	27 ± 7.3	44 ± 3.9	44 ± 17.0
	10.0	31 ± 10.0	33 ± 10.0	36 ± 11.0	31 ± 9.3	Tox	38 ± 15.0
TA	0.0	32 ± 4.9	26 ± 6.6	32 ± 4.9	32 ± 4.7		
	0.5	25 ± 5.4	35 ± 20.0	28 ± 7.4	NT	NT	NT
	1.0	36 ± 12.0	32 ± 3.4	38 ± 6.0	45 ± 14.0	NT	NT
	2.5	24 ± 4.3	62 ± 3.4	41 ± 7.1	62 ± 16.6	NT	NT
	5.0	27 ± 2.9	86 ± 11.0	19 ± 3.7	56 ± 6.6	NT	NT
	10.0	48 ± 4.1	69 ± 37.0	43 ± 4.1	99 ± 18.0	NT	NT
SK	0.0	32 ± 4.9	31 ± 4.5	32 ± 4.9	31 ± 4.5	25 ± 5.7	31 ± 5.8
	0.5	20 ± 4.6	44 ± 28.0	26 ± 8.6	31 ± 5.7	16 ± 0.8	51 ± 32.0
	1.0	19 ± 5.0	47 ± 22.0	21 ± 8.0	53 ± 9.0	20 ± 5.5	65 ± 3.9
	2.5	25 ± 5.2	66 ± 14.0	22 ± 5.7	77 ± 5.9	18 ± 5.1	36 ± 15.0
	5.0	16 ± 3.7	69 ± 23.0	18 ± 4.5	68 ± 0.96	29 ± 10.0	39 ± 14.0
	10.0	NT	37 ± 18.0	29 ± 8.9	65 ± 18.0	28 ± 12.0	31 ± 2.0
JT2	0.0	34 ± 3.5	36 ± 4.8	31 ± 4.3	31 ± 6.6	32 ± 5.3	37 ± 3.7
	0.5	24 ± 8.4	26 ± 9.6	27 ± 8.4	27 ± 7.5	34 ± 10.0	49 ± 2.9
	1.0	25 ± 3.9	39 ± 15.0	30 ± 13.0	41 ± 13.0	32 ± 7.4	65 ± 12.0
	2.5	21 ± 1.6	45 ± 13.0	27 ± 9.6	52 ± 25.0	23 ± 6.2	56 ± 8.8
	5.0	37 ± 8.2	53 ± 8.0	38 ± 7.1	71 ± 18.0	30 ± 7.9	77 ± 13.0
	10.0	47 ± 14.0	77 ± 18.0	33 ± 15.0	77 ± 22.0	37 ± 7.5	55 ± 8.5
Historical Control Values							
None	-	30 ± 8	40 ± 15				
DMSO	0.05	26 ± 6	37 ± 10				
2NF	0.025	2197 ± 445	NT				
BAP	0.005	28 ± 7	609 ± 152				

*NT = Not tested; tox = toxic; None = no additions to top agar; DMSO = dimethyl sulfoxide; 2NF = 2-nitrofluorene; BAP = benzo(a)pyrene.